

**iGEM TU/e 2015**

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## **SDS-PAGE Analysis**

## Table of contents

### SDS-PAGE Analysis

<b>1</b>	<b>SDS-Page Analysis</b>	<b>3</b>
<b>1.1</b>	<b>Preparation of reagents</b>	<b>3</b>
<b>1.1.1</b>	Materials	3
<b>1.1.2</b>	Setup & Protocol	3
<b>1.2</b>	<b>Sample preparation</b>	<b>4</b>
<b>1.2.1</b>	Materials	4
<b>1.2.2</b>	Setup & Protocol	4
<b>1.3</b>	<b>Preparation of the gel</b>	<b>5</b>
<b>1.3.1</b>	Materials	5
<b>1.3.2</b>	Setup & Protocol	5
<b>1.4</b>	<b>Running of the gel</b>	<b>5</b>
<b>1.4.1</b>	Materials	5
<b>1.4.2</b>	Setup & Protocol	5
<b>1.5</b>	<b>Staining of the gel</b>	<b>6</b>
<b>1.5.1</b>	Materials	6
<b>1.5.2</b>	Setup & Protocol	6

# 1 SDS-Page Analysis

## 1.1 Preparation of reagents

**Estimated bench time:** 30 minutes

**Estimated total time:** 30 minutes

**Purpose:** Preparation of buffers and solutions.

### 1.1.1 Materials

- Balance
- Bottle (glassware)
- Bromophenol blue
- dH<sub>2</sub>O
- ddH<sub>2</sub>O
- Glycerol
- Glycine
- DTT
- SDS
- Tris base
- Tris-HCl
- β-mercaptoethanol

### 1.1.2 Setup & Protocol

- For 1 M DTT, the correct amounts are:
  - 2.32 g DTT
  - 15 ml dH<sub>2</sub>O
- For 2X sample buffer SDS-PAGE, the correct amounts are:
  - 1 ml 1M Tris-HCl, pH 6.8
  - 10 ml dH<sub>2</sub>O
  - 2 ml glycerol
  - 2.5 ml β-mercaptoethanol
  - 4 ml 10% SDS
  - 500 µl 1% Bromophenol blue
- For 2X Laemmli buffer, the correct amounts are:
  - 450 µl of 2X sample buffer SDS-PAGE
  - 50 µl 1M DTT
- For 1X SDS running buffer, the correct amounts are:
  - 1.8 l ddH<sub>2</sub>O
  - 2 g SDS
  - 28.8 g glycine
  - 6.04 g Tris base

## 1.2 Sample preparation

**Estimated bench time:** 20 minutes

**Estimated total time:** 45 minutes

**Purpose:** Preparing samples for loading on the SDS-PAGE gel.

### 1.2.1 Materials

- 10  $\mu$ l 2X Laemmili buffer
- 2X Laemmili buffer
- BugBuster<sup>TM</sup> Protein Extraction Reagent
- Culture tube with cells and expressed proteins
- DTT stock (1M)
- Eppendorf tubes
- Heat/shaking block
- MiniSpin Centrifuge
- Tabletop Centrifuge
- Thermal cycler
- Vortex

### 1.2.2 Setup & Protocol

- Spin down the culture tube with cells and expressed proteins for 15 minutes at 3000 xg.
- Discard supernatant.
- Add BugBuster<sup>TM</sup> Protein Extraction Reagent. This needs to be 1/50th volume of the original volume of the culture.
- Shake at 250 rpm at room temperature until the pellet is dissolved.
- Spin down for 10 minutes at 13,400 rpm.
- Transfer the supernatant to a new Eppendorf tube.
- Mix 10  $\mu$ l sample with 10  $\mu$ l 2X Laemmili buffer in a PCR tube.
- Mix by vortexing and short-spin to get the samples at the bottom of the tubes.
- Denature for 5 minutes at 95 °C in the thermal cycler.

## 1.3 Preparation of the gel

**Estimated bench time:** 10 minutes

**Estimated total time:** 10 minutes

**Purpose:** Preparing a SDS-PAGE gel (10%) for loading the samples.

### 1.3.1 Materials

- 1X SDS running buffer
- Precasted SDS-PAGE gel (10%)
- SDS-PAGE kit components:
  - Cell for SDS-PAGE gels
  - SDS-PAGE casting tray
  - SDS-PAGE dummy

### 1.3.2 Setup & Protocol

- Take a cell for SDS-PAGE gels and fill this with 1X SDS running buffer for 50%.
- Take a precasted gel (10%) and take it out of the packing. Remove the strip at the bottom of the gel.
- Take a casting tray and clamp the gel (in the right direction!) and the dummy in this tray.
- Fill the chamber between the gel and dummy completely with 1X SDS running buffer.
- Make sure the chamber is not leaky.

## 1.4 Running of the gel

**Estimated bench time:** 45 minutes

**Estimated total time:** 5 minutes

**Purpose:** Analysis of the protein product with a SDS-PAGE gel.

### 1.4.1 Materials

- Extra-long pipet points
- Precision Plus Protein™ Dual color protein ladder
- Prepared loading samples
- Precasted SDS-PAGE gel (10%)

### 1.4.2 Setup & Protocol

- Load 10 µl of the prepared loading samples on the gel with extra-long pipet points.
- Load 10 µl of Precision Plus Protein™ Dual color protein ladder on the gel.
- Run the gel for 40 minutes on 200 V.

## 1.5 Staining of the gel

**Estimated bench time:** 20 minutes

**Estimated total time:** 2.5 hours

**Purpose:** Analysis of the protein product with a SDS-PAGE gel.

It is essential to work with gloves when working with the staining reagents used.

### 1.5.1 Materials

- Bio-Safe™ Coomassie G-250 stain
- dH<sub>2</sub>O
- ImageQuant
- Rocking plate
- Tray (for transporting the SDS-PAGE gel)
- Tin foil

### 1.5.2 Setup & Protocol

- Carefully take the gel out of the casting frame and put it in a tray.
- Wash the gel with a layer of dH<sub>2</sub>O for 10 minutes on a rocking plate. Cover the tray with tin foil.
- Stain the gel with a layer of Bio-Safe™ Coomassie G-250 stain for 1 hour on a rocking plate. Again, cover the tray with tin foil.
- Discard the Bio-Safe™ Coomassie G-250 stain in the sink.
- Destain the gel with a layer of dH<sub>2</sub>O for 1 hour on a rocking plate.
- Create an image of the gel using the ImageQuant.